

Use of sigma factor M from *Bacillus subtilis* in the development of an orthogonal expression system in *Escherichia coli*.

Indra Bervoets¹, Marjan De Mey², Daniel Charlier¹

¹Research group of Microbiology, Vrije Universiteit Brussel, Belgium

²Centre of Industrial Biotechnology and Biocatalysis, UGent, Belgium

Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning the expression of these synthesis pathways in specific conditions is mandatory for successful production. However, whereas parts and tools have been developed for exponentially growing *Escherichia coli*, these are still lacking for non-growth related production despite clear advantages (in reduction of toxicity and competition, etc). To be able to adequately fine tune a multi-enzyme pathway under such stationary conditions, a new genetic circuit will be developed. This circuit consists of a heterologous sigma factor (σ) recognizing specific promoter sequences, which are not recognized by the native σ factors of *E. coli*. In combination a conditional constitutive promoter library linked to this specific σ factor will be constructed.

Twenty six native promoter sequences transcribed by seven different σ factors from *B. subtilis* were tested for their orthogonality in *E. coli*, by measuring the signal of a red-fluorescent protein engineered downstream of these promoters. On basis of these results we selected *B. subtilis* σ factor M (σ_M), an extracytoplasmatic function (ECF) factor, for further analysis. These ECF proteins offer a number of advantages as their divergence in sequence relative to most other sigma factors, their smaller size and differing consensus sequence. The expression and activity of σ_M will be tested in *E. coli* as well as the efficiency of transcription of *B. subtilis* promoters in *E. coli*. Therefore σ_M has to be able to compete with the natural occurring σ factors for the core *E. coli* RNA polymerase. To obtain a condition specific expression of the *B. subtilis* σ_M in *E. coli*, the corresponding gene can be cloned in the σ_S factor operon of *E. coli*, which is most abundantly expressed in stationary conditions. Combining all these elements should allow us to create an orthogonal genetic circuit that is able to transcribe specific genes under a specific condition with a limited influence on the host cell's metabolism.